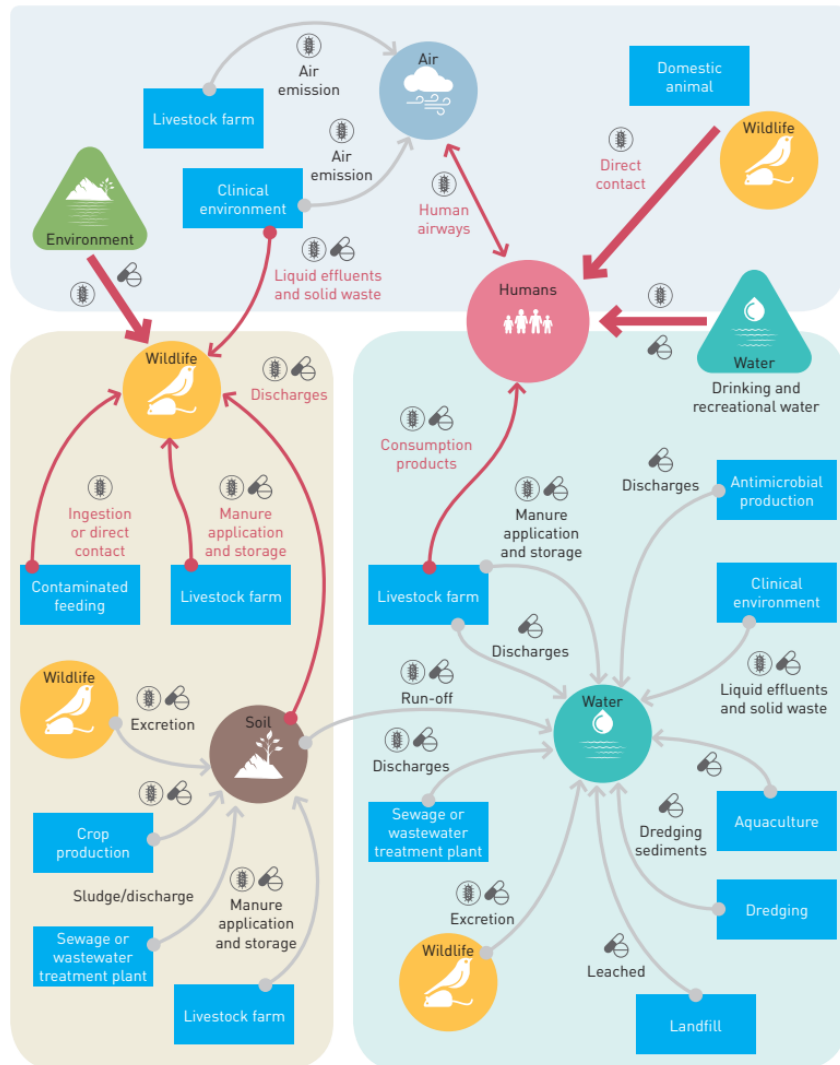


One Health e Resistenza Antimicrobica (AMR)



La Dimensione Ambientale dell'AMR



References

Resistant microorganisms Antimicrobial residue Activities Environmental aspects

Figure 4

L'ambiente naturale (acqua/suolo/aria) può essere un importante serbatoio e fattore trainante della resistenza antimicrobica.

Principali contribuenti al fenomeno includono:

- Acque reflue urbane e ospedaliere
- Effluenti della produzione farmaceutica
- Fenomeni di run-off (agricoltura e zootecnia)
- Altri rifiuti e rilasci

“Per comprendere lo sviluppo, la trasmissione e la diffusione di AMR nell'ambiente, è imperativo considerarlo attraverso una prospettiva multidimensionale”

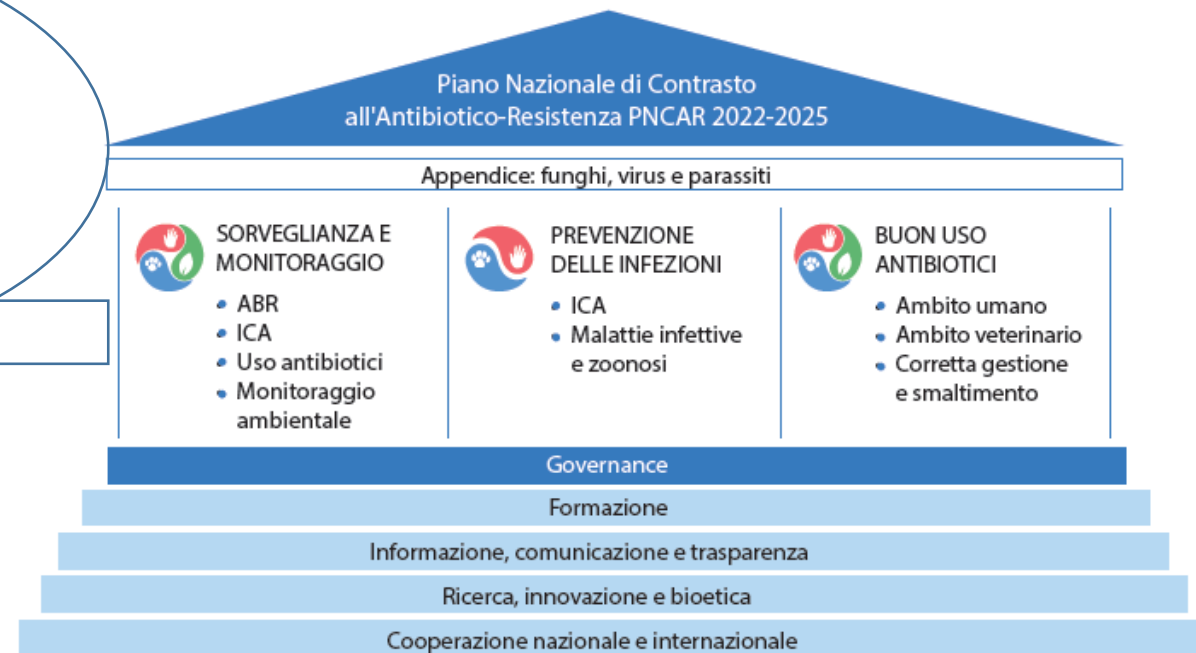
PNCAR

Piano Nazionale di Contrasto all'Antibiotico Resistenza 2022-2025

Obiettivo: fornire le linee strategiche e le indicazioni operative per affrontare l'emergenza dell'AMR nei prossimi anni, seguendo un approccio multidisciplinare e una visione *One Health*



Sorveglianza e monitoraggio integrato dell'antibiotico-resistenza (ABR), dell'utilizzo di antibiotici, delle infezioni correlate all'assistenza (ICA) e **monitoraggio ambientale**.

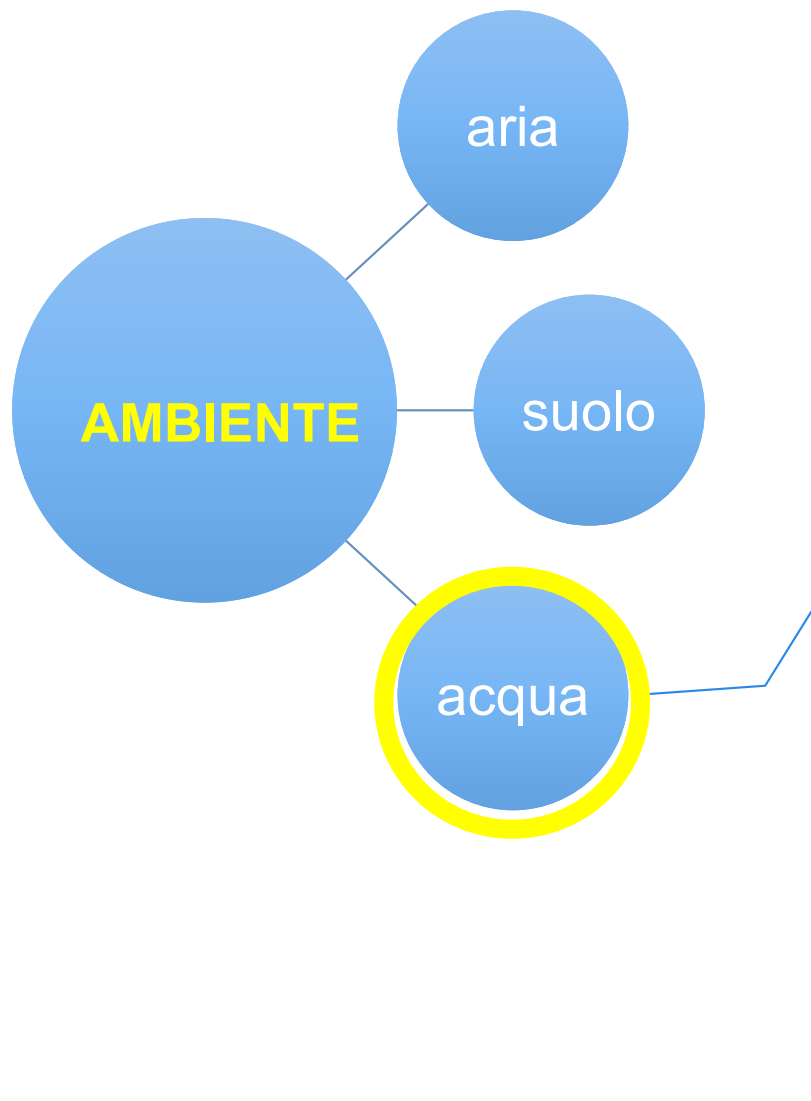


AMR nei Comparti Ambientali



Focus sull'Acqua


AZIONI PREVISTE DAL PNCAR 2022-2025 PER IL MONITORAGGIO AMBIENTALE DEGLI ANTIBIOTICI E DELL'AMR



FOCUS SULL'AMBIENTE ACQUATICO

- Ricerca di AMR e di antibiotici in reflui urbani e in acque superficiali
- *Monitoraggio scarichi ad elevato contenuto ARGs, antibiotici e patogeni mediante accordi con i produttori (azione centrale)*

Revisione della Direttiva EU sulle Acque Reflue

 European Parliament

Rafforzate le norme per il trattamento e il riutilizzo delle acque reflue urbane | Attualità | Parlamento europeo

Mercoledì, i deputati hanno approvato in via definitiva nuove norme per la raccolta, il trattamento e lo scarico delle acque reflue urbane.

Apr 10, 2024



European Parliament

2019-2024



TEXTS ADOPTED

P9_TA(2024)0222

Urban wastewater treatment

European Parliament legislative resolution of 10 April 2024 on the proposal for a directive of the European Parliament and of the Council concerning urban wastewater treatment (recast) (COM(2022)0541 – C9-0363/2022 – 2022/0345(COD))

Article 1. Subject matter

This Directive lays down rules on the **collection, treatment, and discharge of urban wastewater, to protect the environment and human health, in line with the One Health approach**, while progressively reducing greenhouse gas emissions to sustainable levels, improving the energy balance of urban wastewater collection and treatment activities and contributing to the transition towards a circular economy. It also lays down rules on access to sanitation for all, on transparency of the urban wastewater sector, **on the regular surveillance of public health relevant parameters in urban wastewaters** and on the implementation of the polluter-pay principle.

Revisione della Direttiva EU sulle Acque Reflue

Riciclo e rischi associati

Article 15. Water reuse and discharges of urban wastewater

Member States shall systematically **promote the reuse of treated wastewater** from all urban wastewater treatment plants where appropriate, especially in water-stressed areas, and for all appropriate purposes. (...)

Article 18. Risk assessment and management

By 31 December 2027, Member States shall **identify and assess the risks caused by urban wastewater discharges to the environment and human health**, (...)

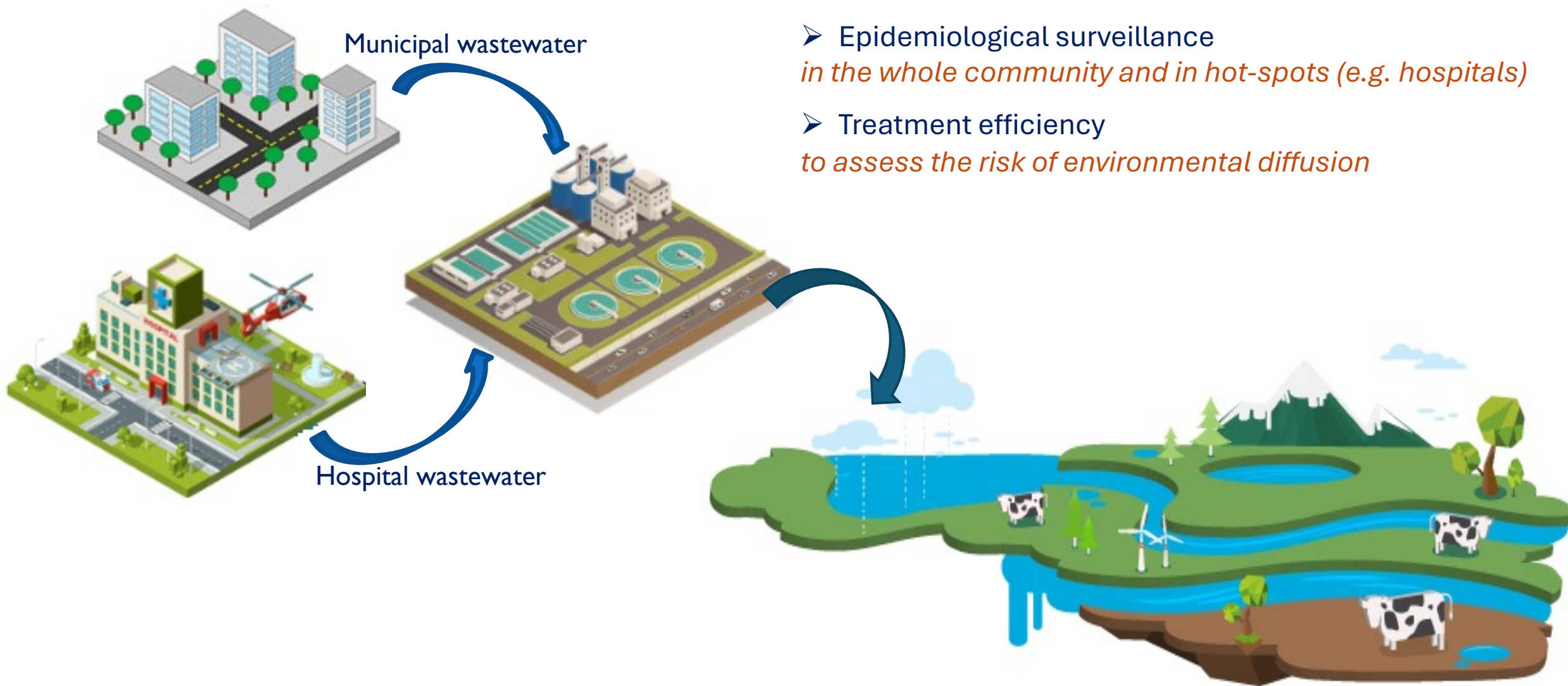
Sorveglianza epidemiologica

Article 17. Urban wastewater surveillance

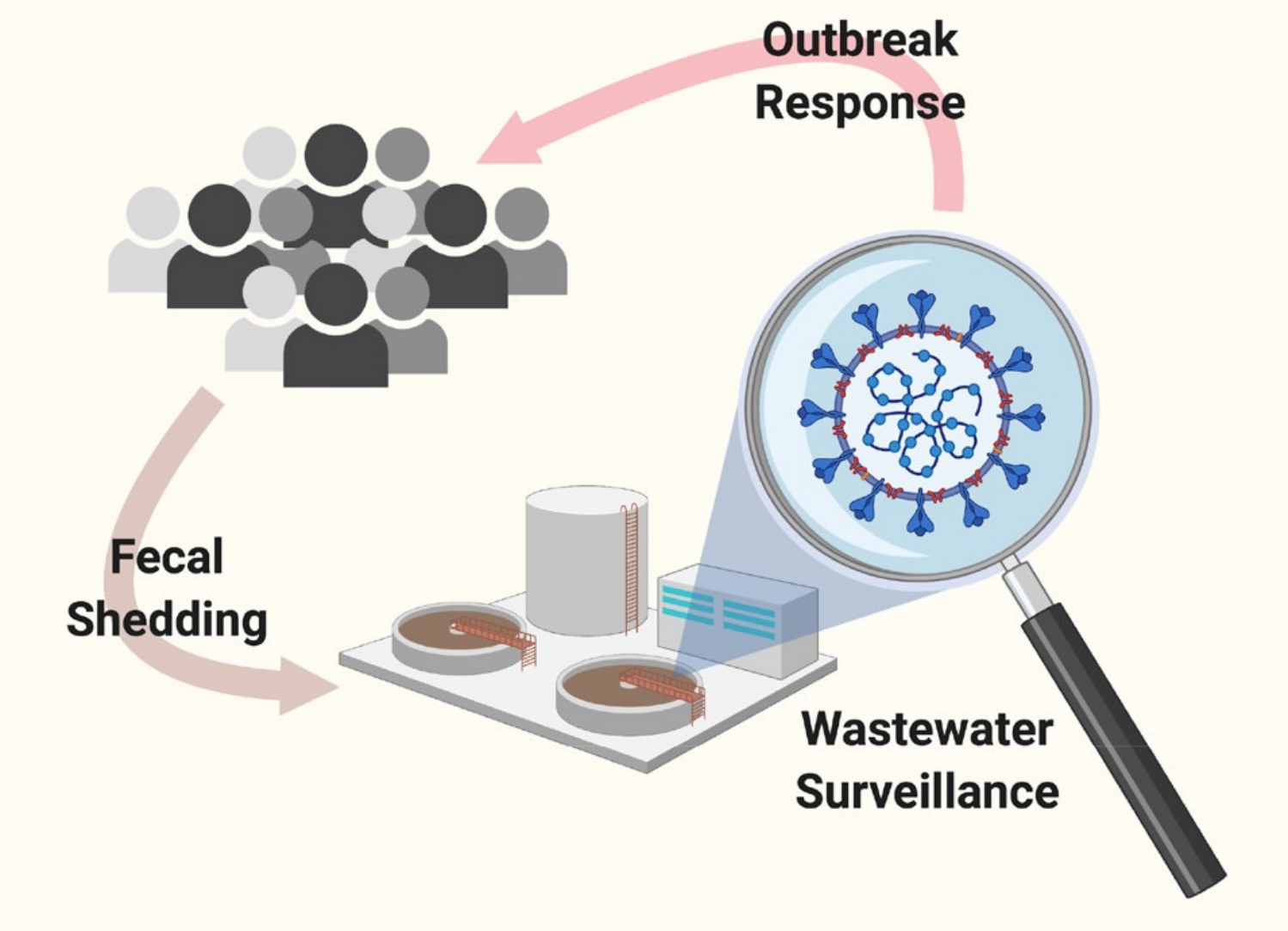
Member States shall **set up a national system** for cooperation and coordination between competent authorities responsible for public health and competent authorities responsible for urban wastewater treatment **with regard to the identification of relevant public health parameters, that are to be monitored at least in the inlet of urban wastewater treatment plants**, taking into account available recommendations by the European Center of Disease Prevention and Control (ECDC), by the Health Emergency Preparedness and Response Authority (HERA) and the World Health Organisation (WHO) among others and including for instance those in the following **list: SARS-CoV-2 virus and its variants; poliovirus; influenza virus; emerging pathogens; any other public health parameters that are considered relevant by the competent authorities of the Member States for monitoring**; (...)

For agglomerations of 100 000 p.e. and above, Member States shall, (...) **ensure that antimicrobial resistance is monitored in urban wastewater**. The Commission shall adopt implementing acts (...) to ensure a uniform application of this Directive by **establishing a minimum frequency of sampling and a harmonised methodology for measuring antimicrobial resistance in urban wastewaters**, (...)

Acque Reflue: Duplice Aspetto



Wastewater-Based Epidemiology (WBE)



WBE: Aspetti Metodologici

Campionamento

Processamento

Ricerca e Quantificazione del Target



N.A. Extraction



qPCR Assay

Detection and quantification of specific known genes (pathogen or trait specific)



NGS

Shotgun sequencing of all genes in the sample (metagenome, virome, resistome)

WBE Durante la Pandemia

WASTEWATER BASED EPIDEMIOLOGY



1

Infection by SARS-CoV-2 and shedding of the virus to the stool begins

2

Sampling of the sewer and transport to laboratory where analysis is carried out

3

Result of the wastewater analysis

4

Symptom onset of the infected individual

5

Positive PCR result from the nasopharyngeal swab

6

Hospitalization

7

Death

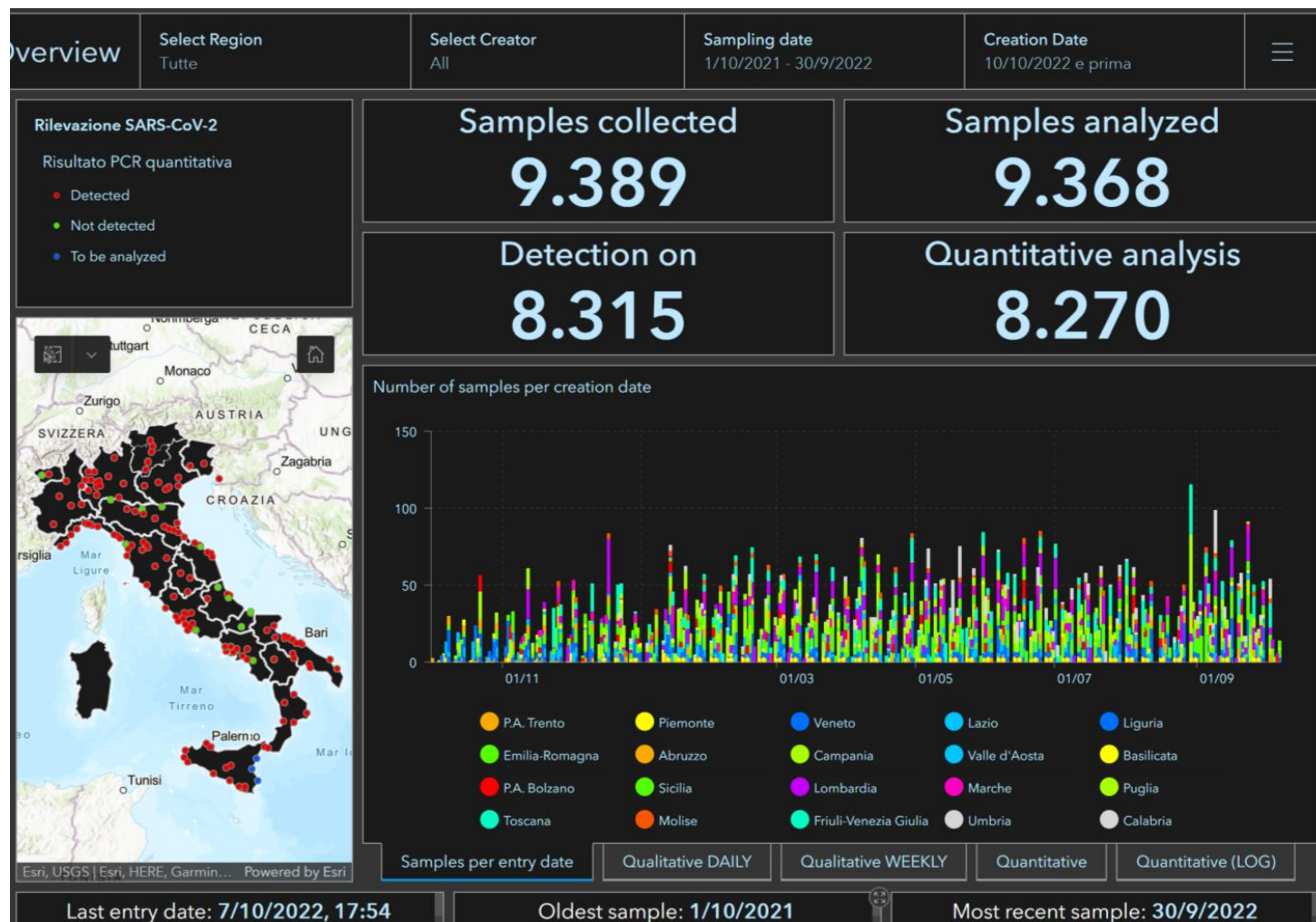
12-13 Days

26-27 days

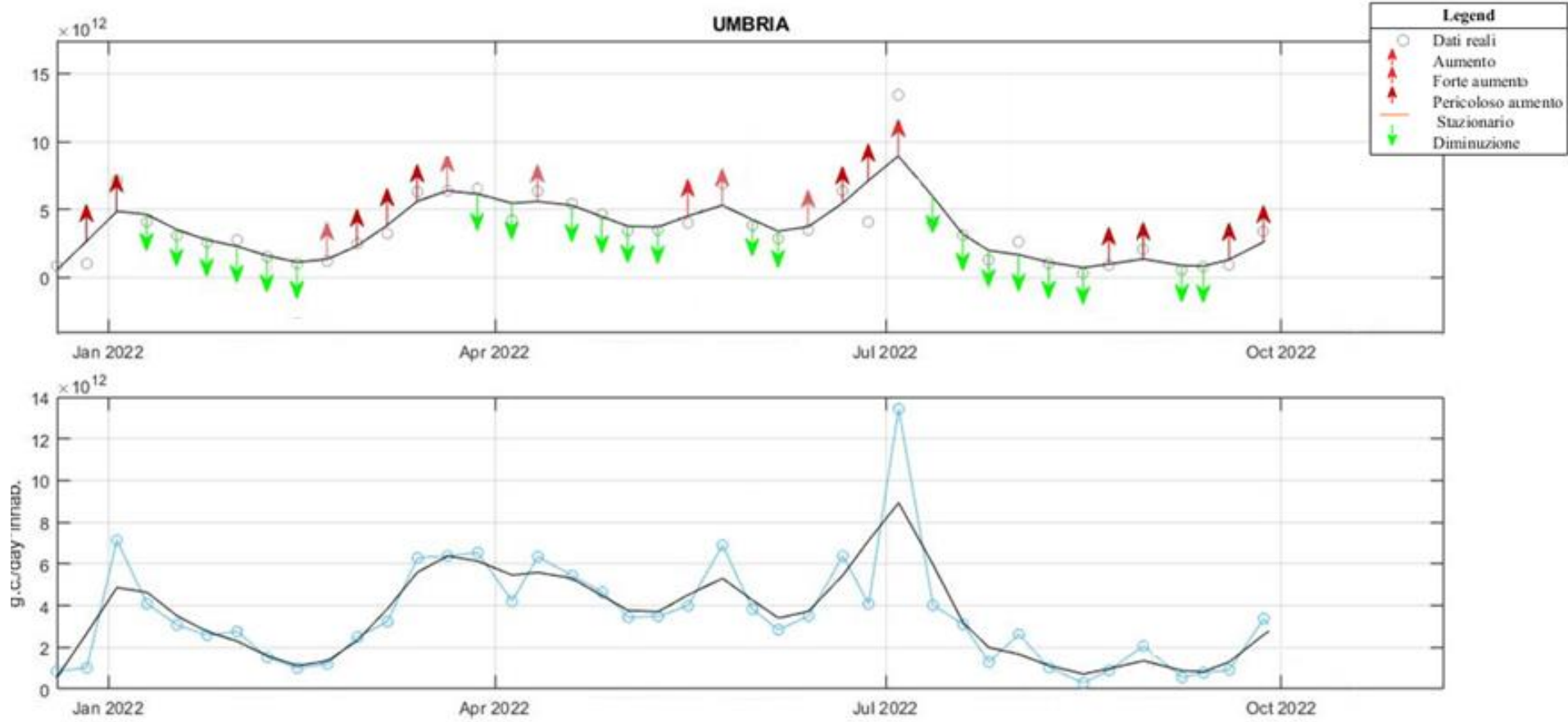
IL Network SARI

Sorveglianza Ambientale Reflue in Italia

- Coordinamento tecnico-scientifico dell'**Istituto Superiore di Sanità**
- **167 punti di campionamento** (75 gestori)
- **43 Laboratori** (Università, ARPA, IZS, Lab. Servizio)
- **Regione Umbria**, Servizio Prevenzione, Sanità veterinaria, Sicurezza alimentare
- **UniPG** (Analisi, Elaborazioni Dati, Interazione con ISS)

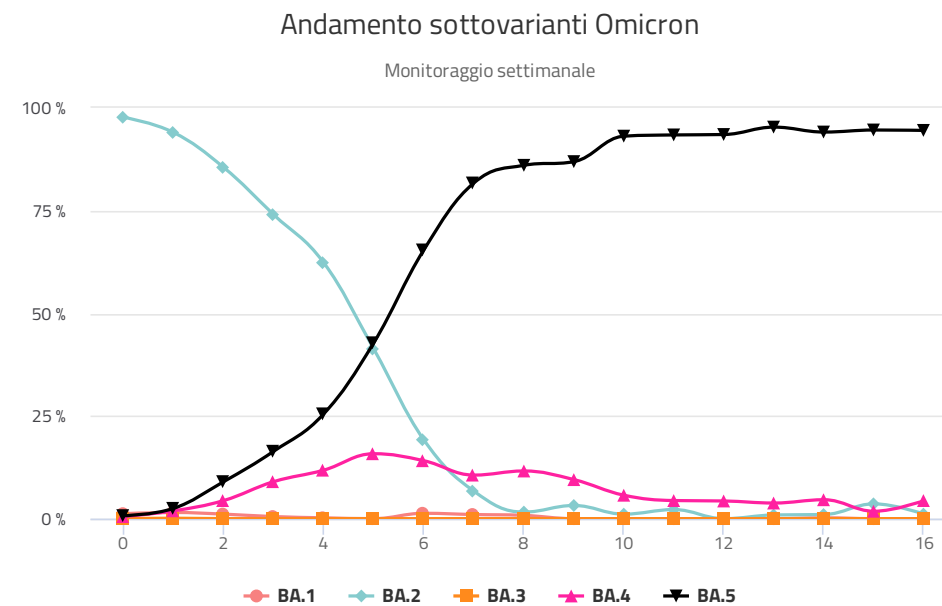
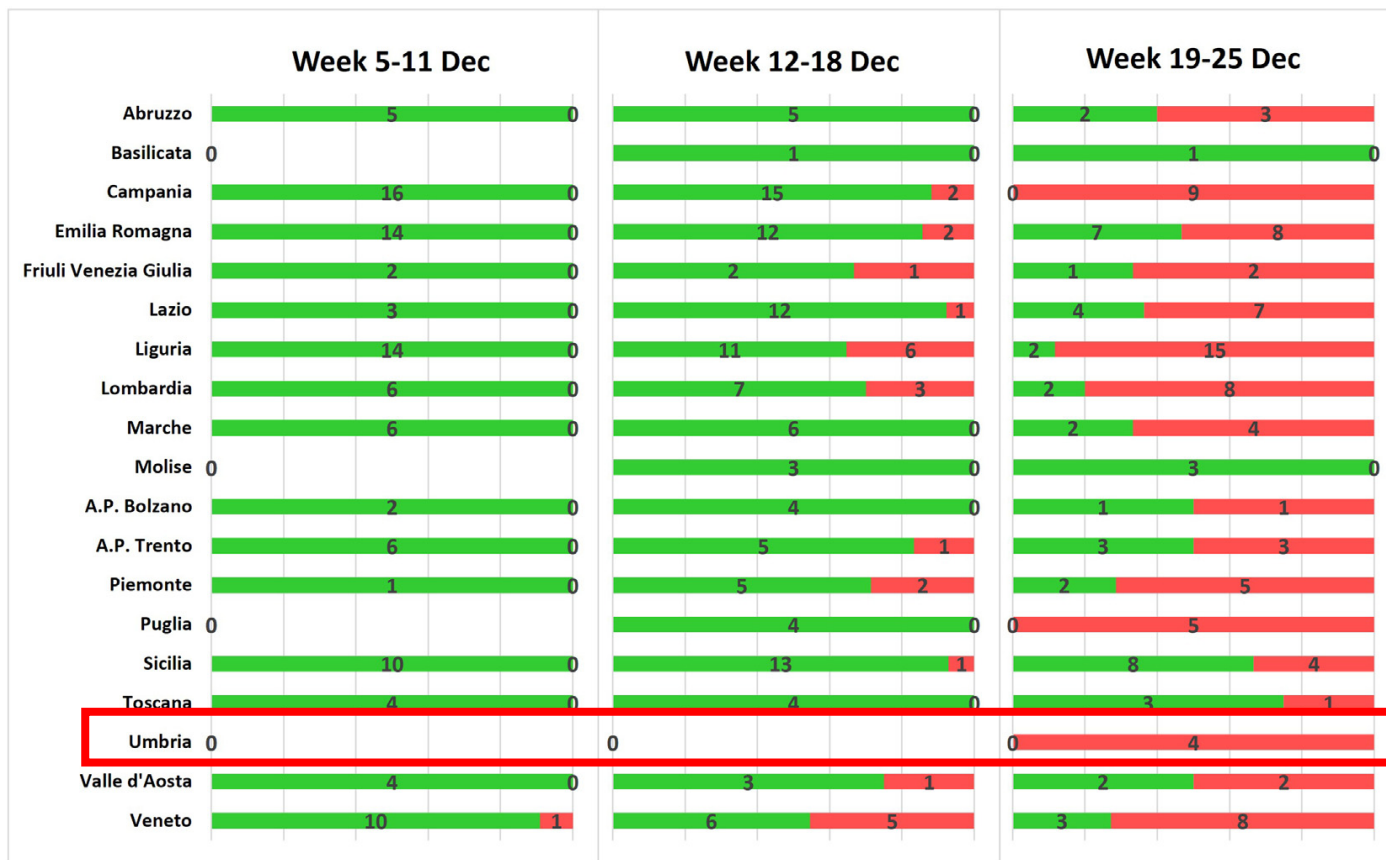


SARS-CoV-2 nelle A.R. in Umbria



SARS-CoV-2 nelle A.R. in Umbria

Insorgenza Variante Omicron (fine 2021)



SARS-CoV-2 nelle A.R. in Umbria

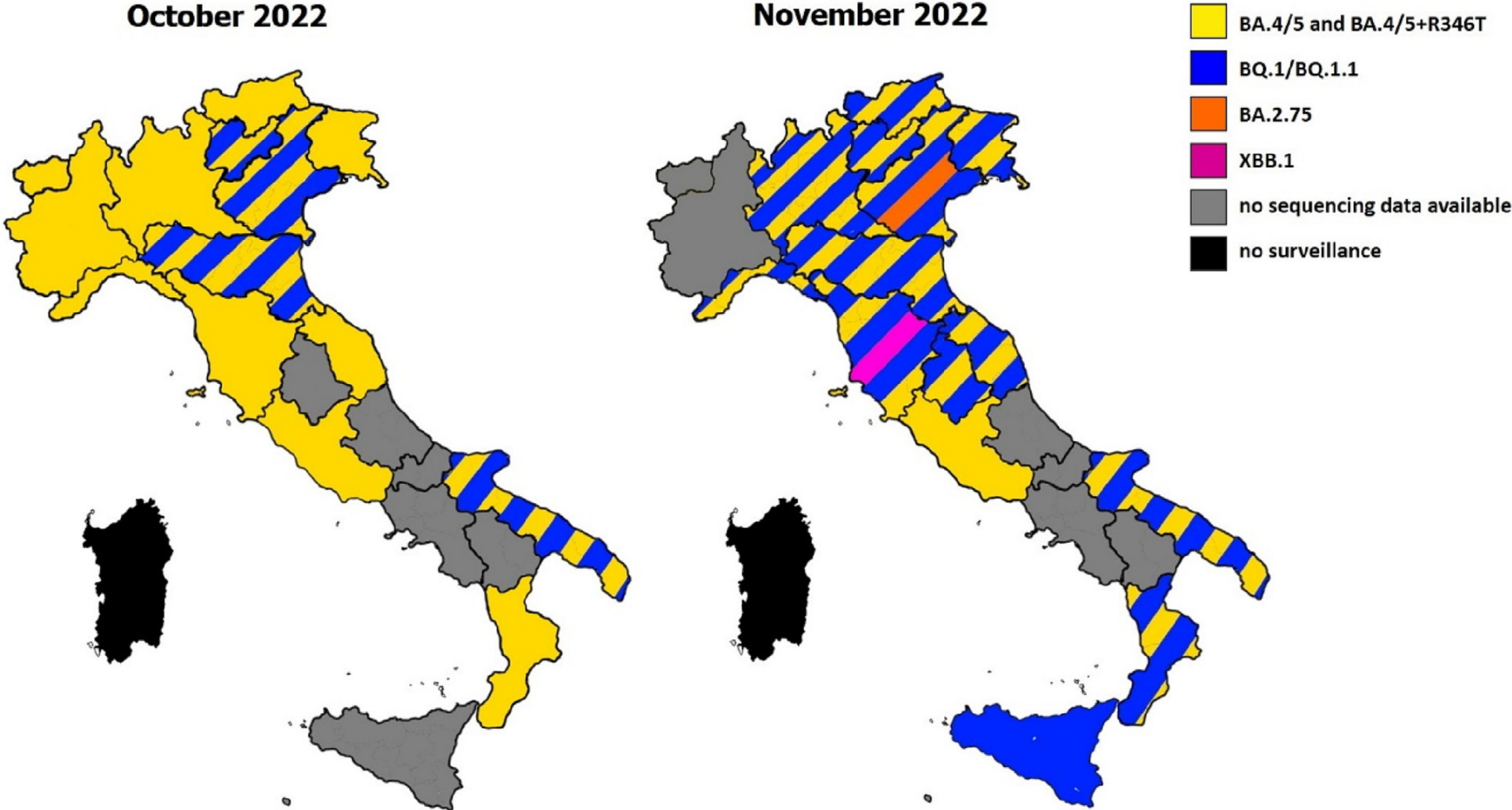
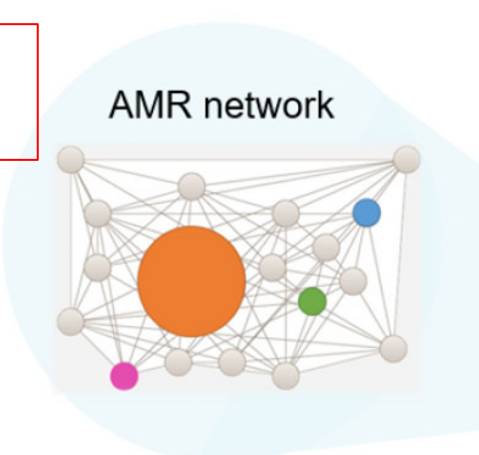


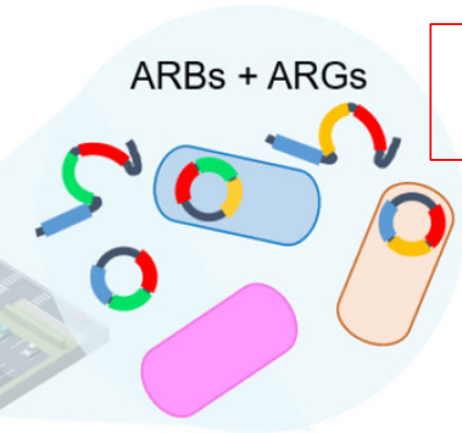
Fig. 1. Omicron lineages/sublineages detected by NGS in October and November 2022.

AMR nelle Acque Reflue

Sorveglianza Ambientale



Valutazione del rischio di diffusione ambientale



Sewage



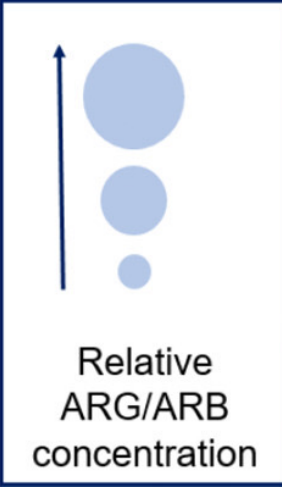
Wastewater treatment



Effluent



Natural environment

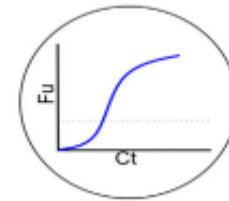
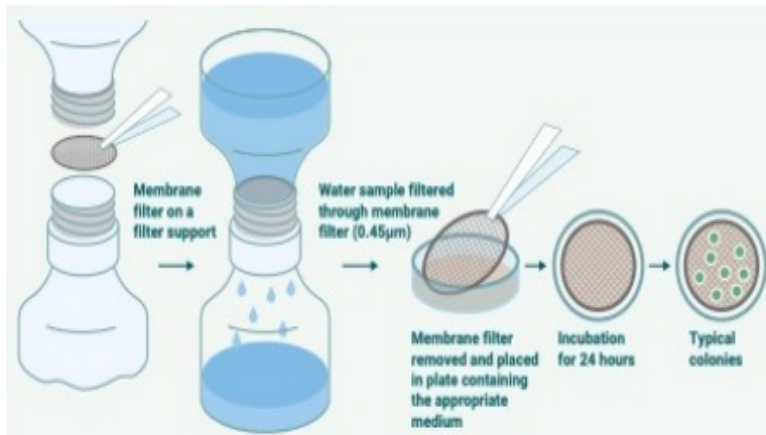


AMR nelle Acque Reflue: Approccio



Cultural approach

Molecular approach



AMR nelle Acque Reflue: Network SARI

Tavolo Interregionale per l'implementazione del Piano Nazionale di Contrasto all'Antimicrobicoresistenza (PNCAR 2022-2025) sezione ambiente- 26 gennaio 2024 sull'utilizzo del protocollo.



- ❖ **Consenso generale** sull'utilizzo del Tricycle protocol come **primo protocollo condiviso a livello nazionale**
- ❖ Estensione ai metodi molecolari (quantificazione di geni di resistenza) entro la fine del PNCAR

Metodi colturali (Tricycle protocol)

Metodi molecolari (ricerca e quantificazione geni)

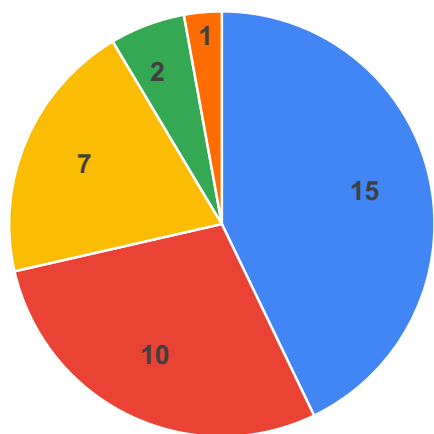
**PNCAR
2026 - 2029**

AMR nelle Acque Reflue: Network SARI

79 Partecipanti

(i.e. seguono le discussioni e rivedono i documenti prodotti)

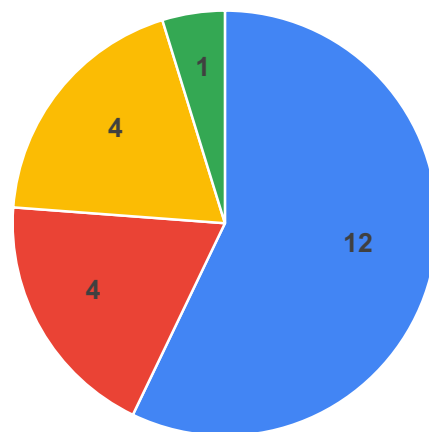
35 diversi Enti/Istituzioni



40 Contributori

(i.e. partecipano attivamente alla stesura dei documenti/protocolli, ricercando documenti, fornendo dati sperimentali, etc)

21 diversi Enti/Istituzioni



Gruppo di Lavoro *Geni di Antibiotico Resistenza*

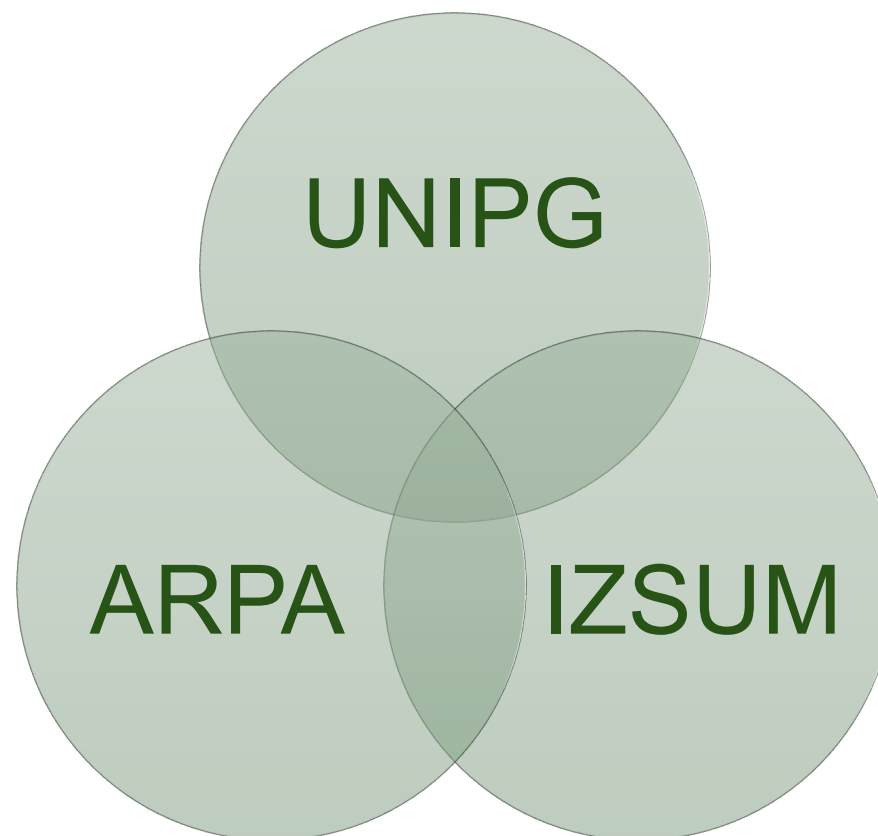
Obiettivo del GdL è quello di sviluppare e validare, entro la fine del PNCAR, un **protocollo di sorveglianza nei reflui** basato su metodi molecolari di quantificazione di **geni di resistenza agli antibiotici**

PNCAR in Umbria

PNCAR 2022-2025

Gruppo Tecnico di Coordinamento e Monitoraggio Regionale

Tavolo Tecnico Ambientale



Progetto Pilota: Target Genici



*qPCR assays to quantify
AMR genes*

Target Selection and qPCR Assay Development:

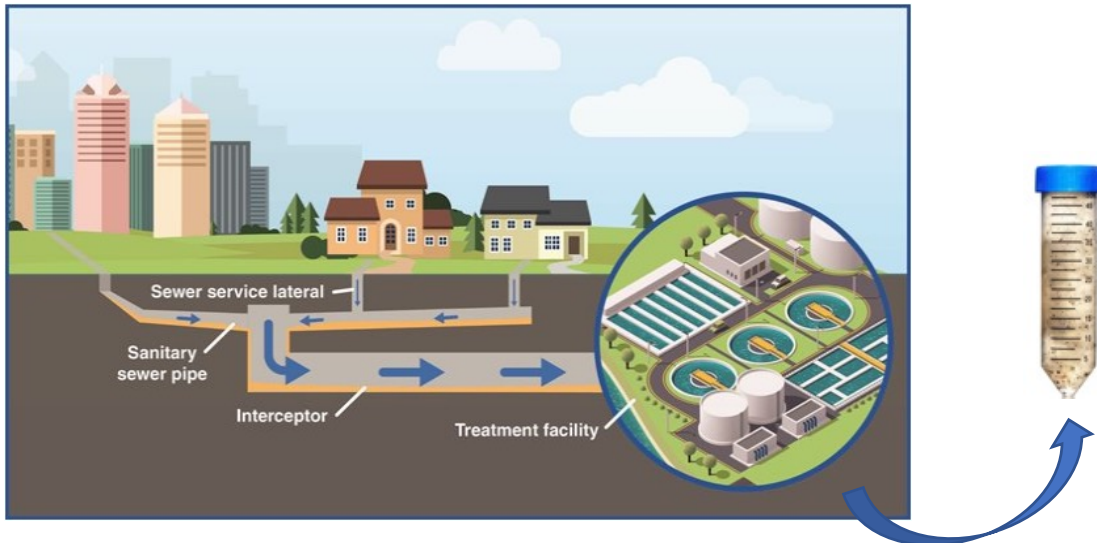
- **β -lactam resistance: blaCTX-M ;**
relevant for multi-resistance due to Extended-Spectrum Beta-Lactamases (ESBLs), including third-generation cephalosporin-resistant *E. coli* (CREC) (*)
- **Carbapenems resistance : blaKPC;**
relevant for carbapenemase-resistant *K. pneumoniae* (CRKP) (*)
- **Vancomycin resistance: vanA/vanB;**
relevant for vancomycin-resistant *E. faecium* (VRE-faecium) (*)
- **Methicillin resistance: mecA/mecC;**
relevant for methicillin-resistant *S. aureus* (MRSA) (*)
- **Sulphonamide resistance: sul1;**
widespread, an anthropogenic and gene transfer indicator
- **Tetracycline resistance: tetA;**
widespread and anthropogenic indicator
- **Mobile genetic elements: int11;**
it promotes gene transfer, an anthropogenic indicator, can be associated with multi-antibiotic resistance
- **Total bacteria: 16S rRNA;**
total bacteria quantification, normaliser for target gene quantification

(*) Main pathogen/antibiotic combinations under AR-ISS surveillance in Italian regions and under observation at European level by the ECDC

Progetto Pilota: Depuratori

Acque Reflue Urbane:

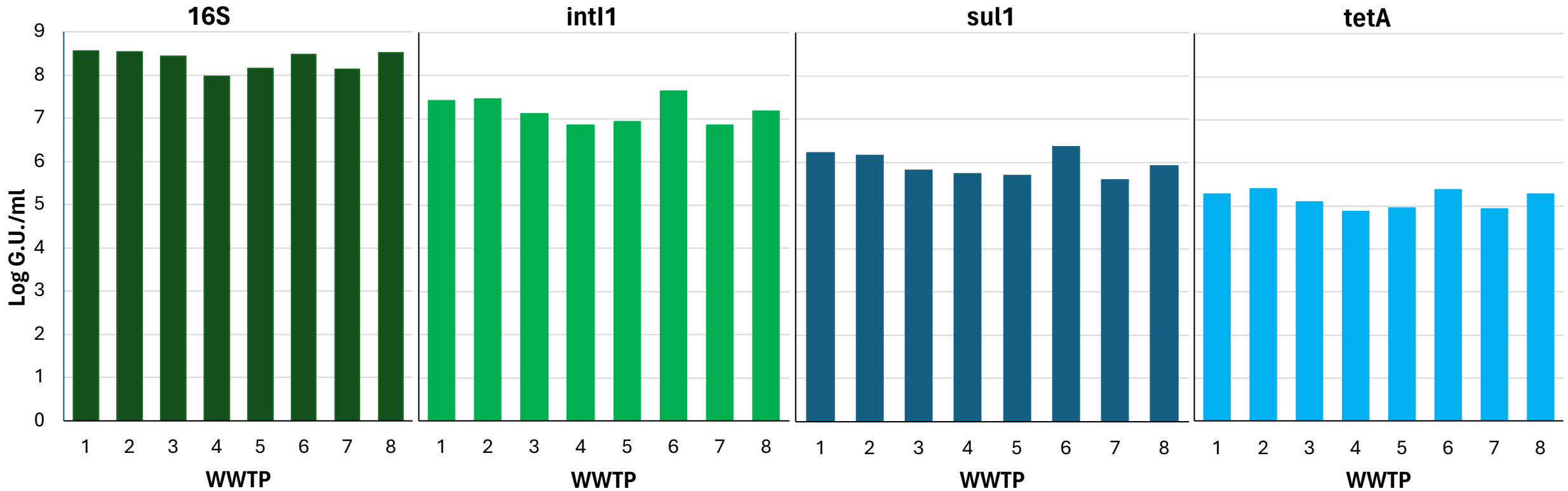
- ✓ Collettore Impianto Trattamento Reflui
 - *Monitoraggio AMR nell'intera popolazione*
- ✓ Inlet vs. Outlet Impianto Trattamento Reflui
 - *Valutazione dell'efficacia del trattamento e del rischio di diffusione ambientale di AMR*



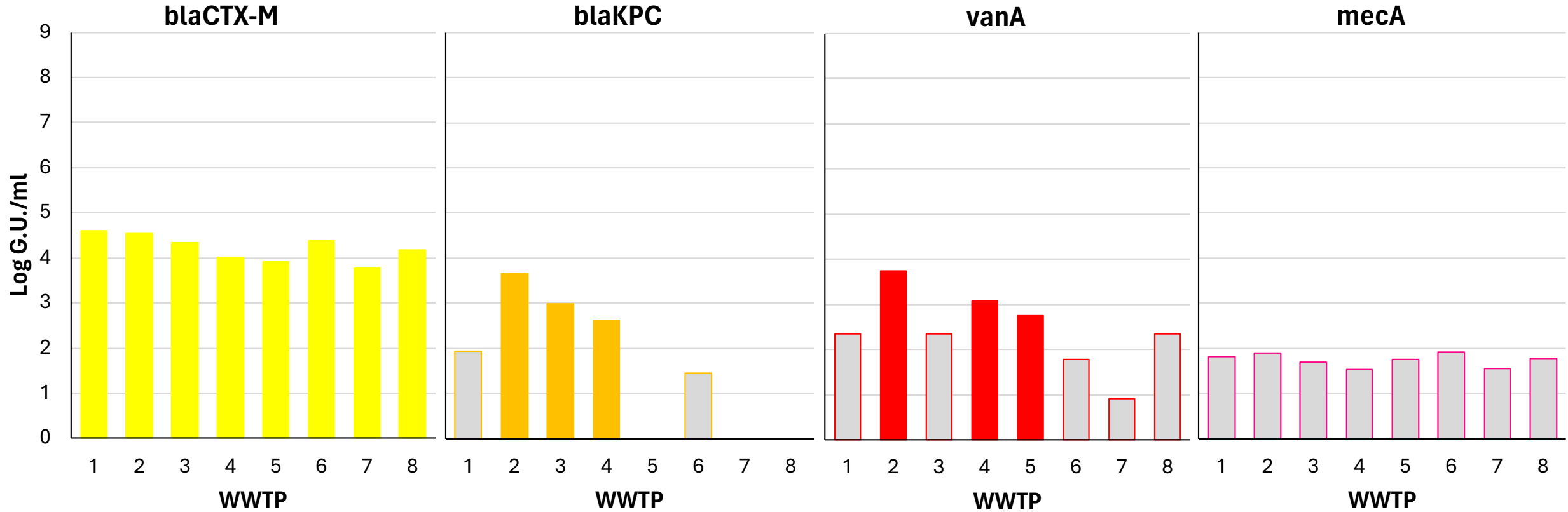
WWTP SAMPLES

WWTP	Description	P.E.
1	Large city	120000
2	Large city with Hospital	100000
3	Large city	95000
4	Large city	90000
5	Rural Area	7000
6	Rural Area	5000
7	Rural Area	3500
8	Rural Area	1000

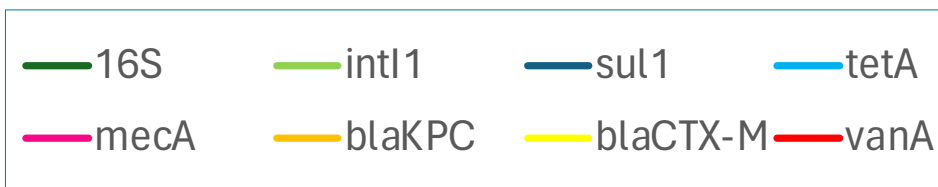
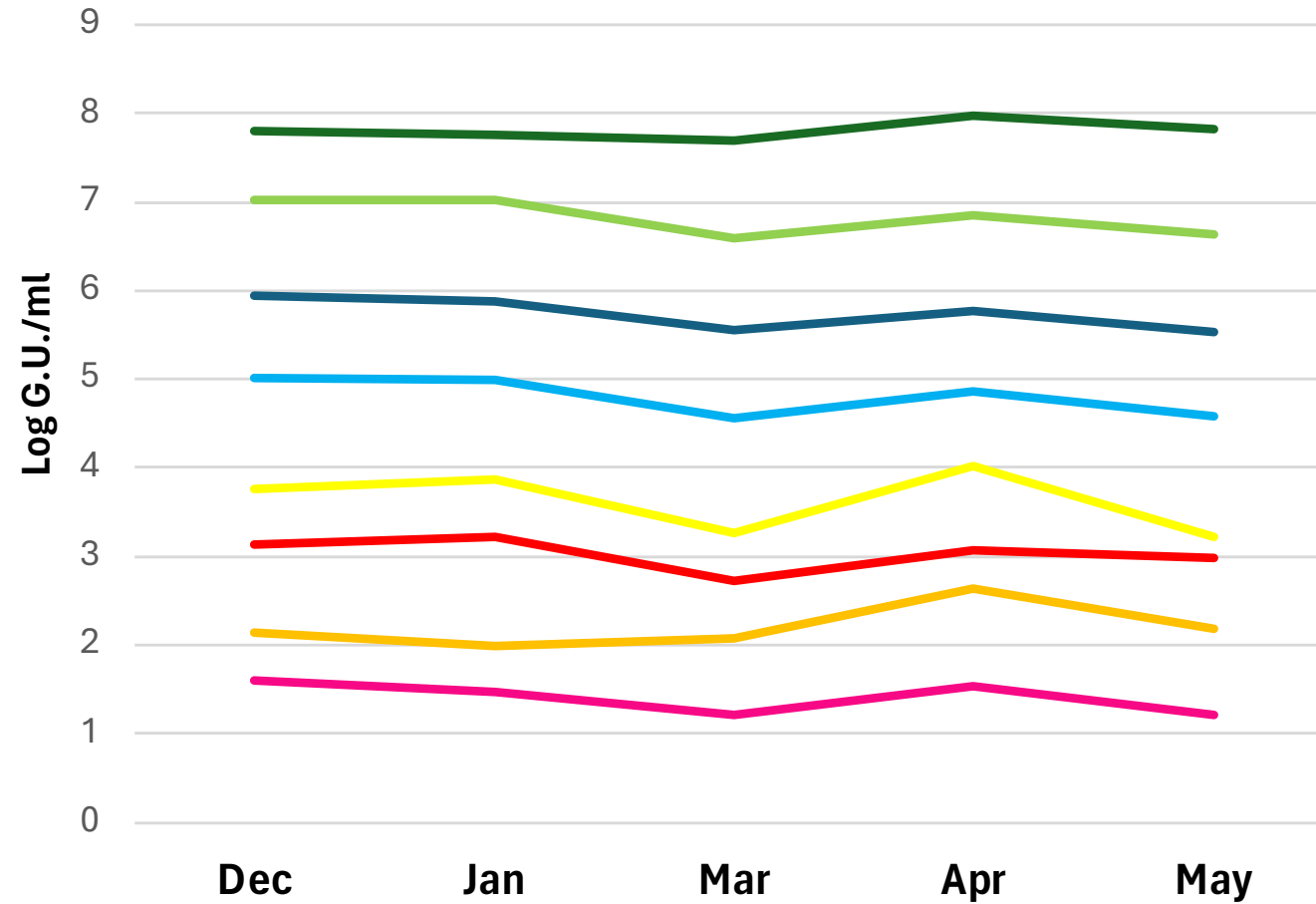
Progetto Pilota: Reflui in Entrata



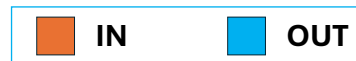
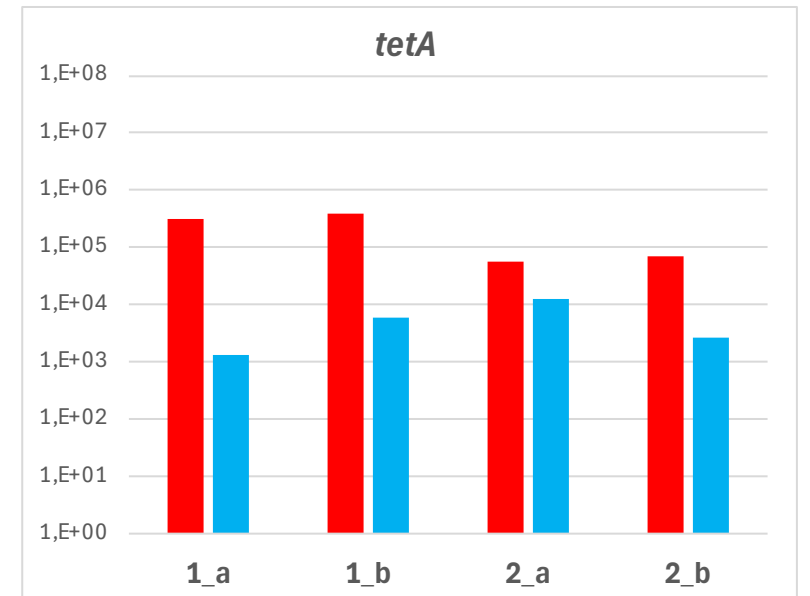
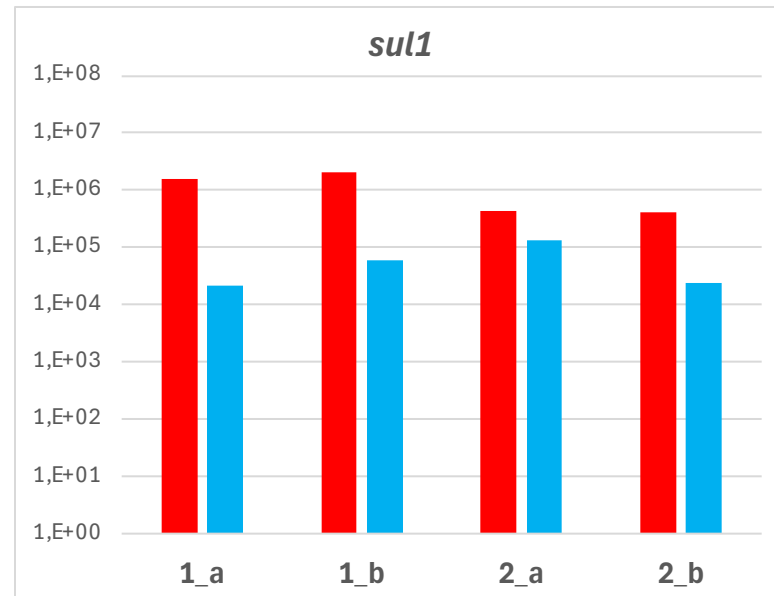
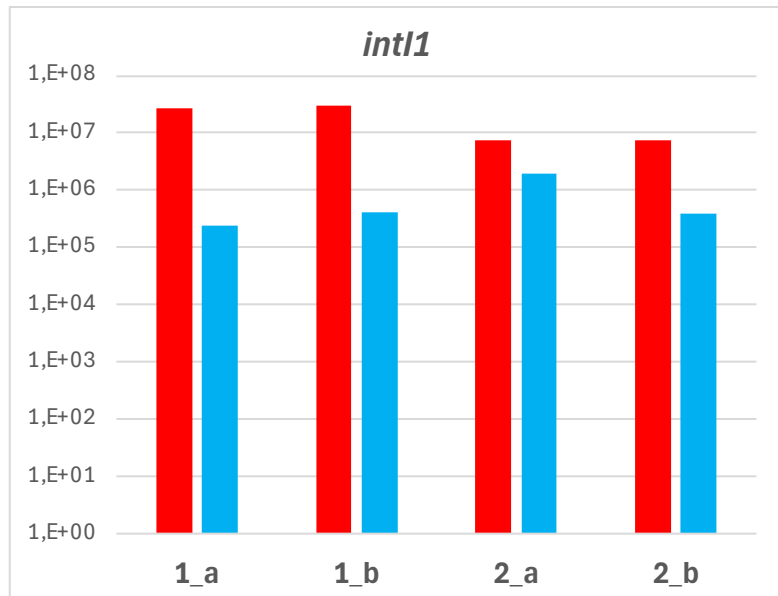
Progetto Pilota: Reflui in Entrata



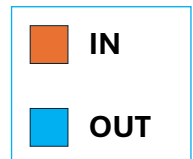
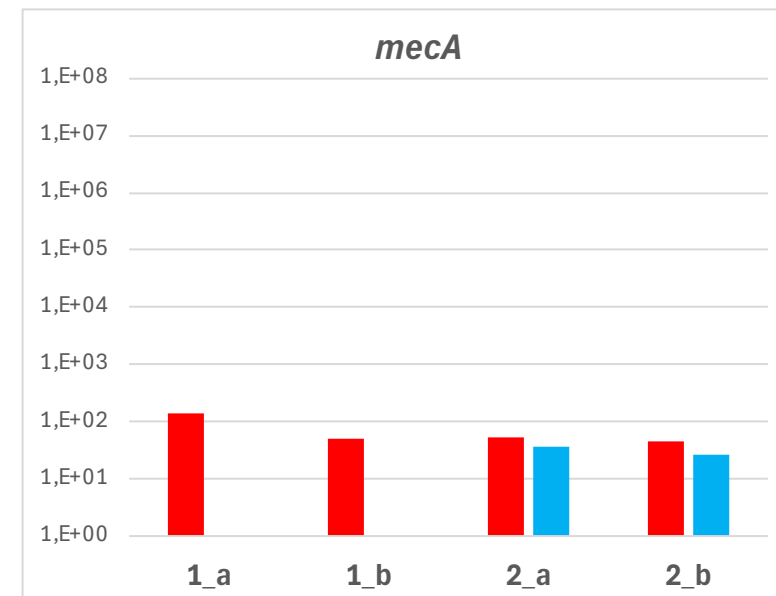
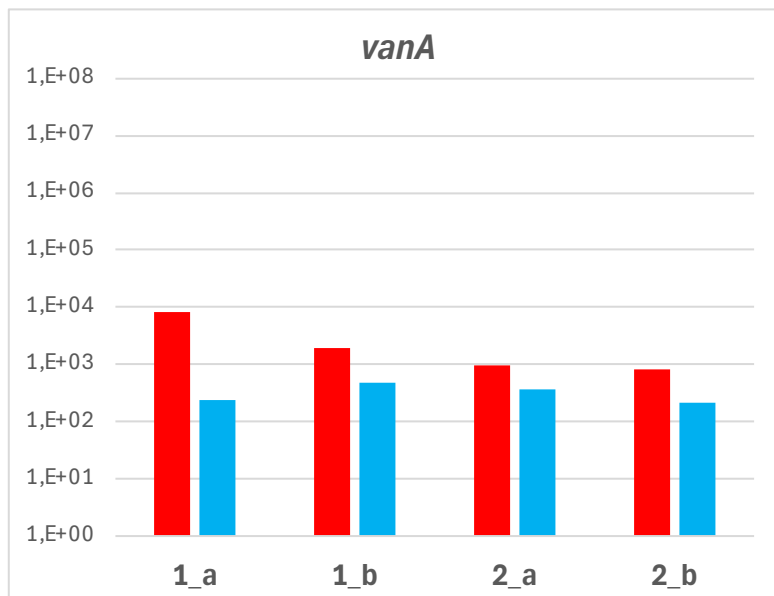
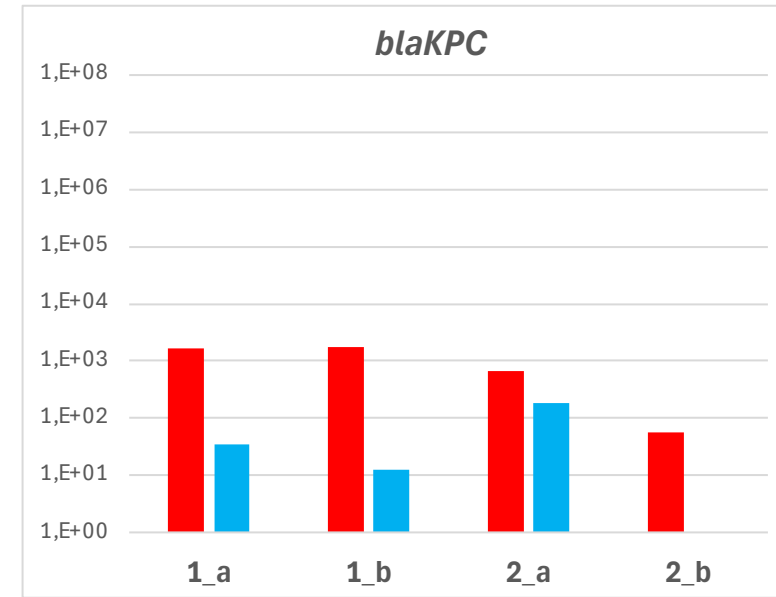
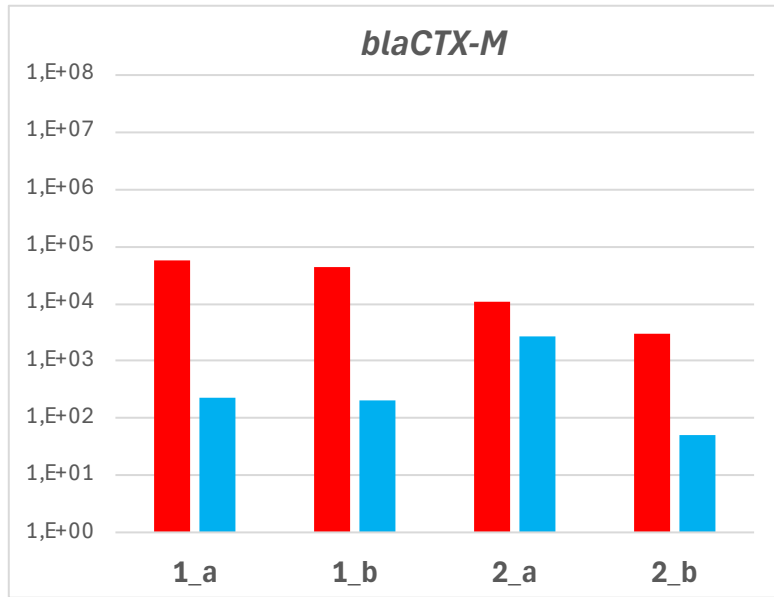
Progetto Pilota: Reflui in Entrata



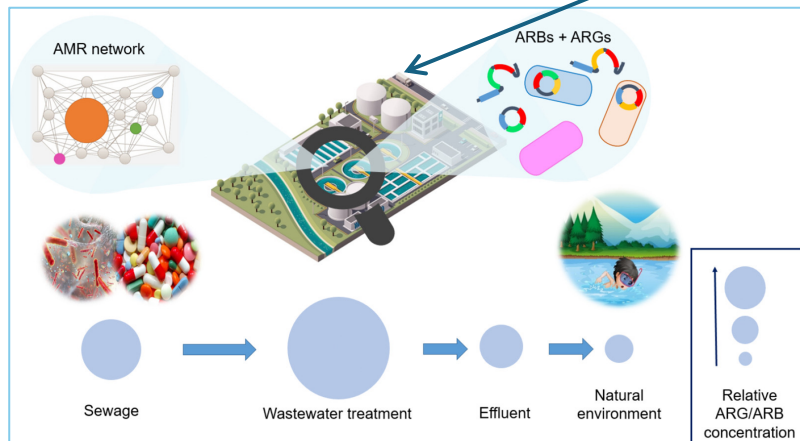
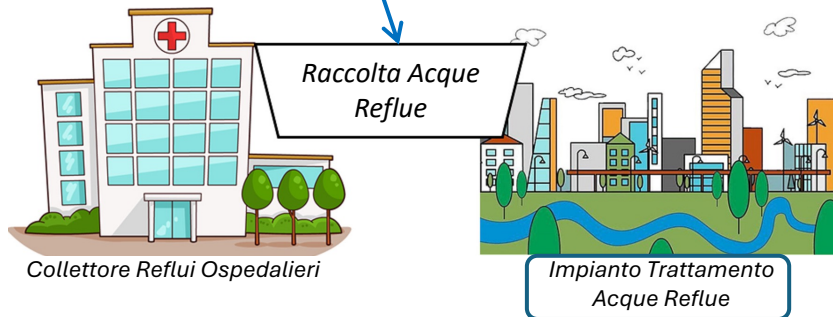
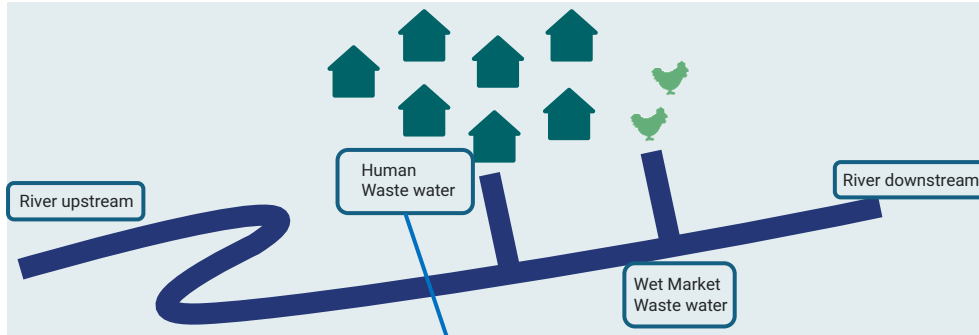
Progetto Pilota: Reflui in Entrata vs Uscita



Progetto Pilota: Reflui in Entrata vs Uscita



PNCAR in Umbria: Prospettive



Acque Reflue Urbane:

- ✓ Collettore Impianto Trattamento Reflui
 - *Monitoraggio AMR nell'intera popolazione*
- ✓ Inlet vs. Outlet Impianto Trattamento Reflui
 - *Valutazione dell'efficacia del trattamento e del rischio di diffusione ambientale di AMR*
- ✓ Collettore Reflui Ospedalieri
 - *Monitoraggio AMR in "hot spot"*

Altri Campioni Ambientali:

- ✓ Acque Superficiali
 - *Valutazione della diffusione ambientale di AMR*
- ✓ Reflui Impianti Zootecnici
 - *Informazioni per PNCAR ambito animale*
- ✓ AMR nei pesci
 - *Progetto Ricerca con IZSUM*